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ORIGINAL ARTICLE

Effect of Humic Acid Application on Qualitative Characteristic and Micronutrient Status in *Petunia hybrid* L.

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ABSTRACT
Humic acid (HA) is a relatively stable product of organic matter decomposition and thus accumulates in environmental systems. The aim of this study was to monitor the effect of foliar application of humic acid on growth characteristics of Petunia hybrid L; such as fresh and dry weight of root and shoot, height, tillers and flowers, Leaf area index(LAI),chlorophyll content, relative water content(RWC) and absorption of micronutrient(Fe, Zn, Cu, Mn). A pot experiment was conducted in a completely randomized design with five treatments of HA (0, 100, 300, 600 and 900 ppm) and three replications. Data were analyzed by SPSS software and Duncan test. Results showed that increase in humic acid concentration, increased all evaluated growth traits and micronutrient absorption compare than control at statistical level of 1% and 5%, significantly.
Key word: humic acid, foliar application, growth characteristics, micronutrient

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INTRODUCTION

Petunia hybrida is an ornamental plant because of ease of breeding, low expectations and having beautiful and very diverse colors is one of the most common flowers in green spaces of municipal. Petunia is a tropical plant with annuals and perennials varieties of Solanaceae family [1], and planted in outdoors and pots. It's requiring to full light of the sun in order to optimal growth and introduce bright color flower [2]. Different research have shown that ornamental plant nutrition during the growth stage in greenhouse (Transplanting stage) not only preventing nutritional deficiency and toxicity but also improve qualitative and quantitative characteristic [3].

Foliar feeding of nutrients has become an established procedure in increase yield and improve the quality of crop products [4].This procedure improves nutrient utilization and lower environmental pollution through reducing the amount of fertilizers added to soil. Foliar application of of nutrients may actually promote root absorption of the same nutrient or other nutrients through improving root growth and increasing nutrients uptake [5].

Organic fertilizer production from agricultural waste on the one hand help to restore the natural resources and reduce pollution of water and soil, on the other hand is reducing fossil energy consumption. The materials such as compost, manure and humic acid will increase plant growth. Humic acid is a natural polymer containing carboxyl and phenolic positions to do exchange process. Humic acid is a result of chemical and biological reactions of soil as Humification and with the molecular weight of 3,000 to 30,000 Daltons form stable and soluble complexes with micronutrient[6]. Humic acid by improving nutrient uptake and effects on hormones can stimulate the plant growth. Recently, among the fertilization strategies, the foliar spray with different molecules as humic acid has been introduced. These organic substances have no harmful threat to the quality of the environment [7]. Under water stress, foliar fertilization with humic molecules increased leaf water retention and the photosynthetic and antioxidant metabolism [8]. Foliar spray with humic acid also increased root length [9] and leaf area index [10]. Research studies showed that humic acid can be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance [11].

Zn is the second most abundant transition metal after iron (Fe) and is involved in various biological processes in organisms [12]. Final production (quantity and quality) is one of the main characteristics that should be evaluated in studies concerning plant crops [13]. Zinc (Zn) is an essential element for plant that act as a metal component of various enzymes or as a functional structural or regulatory cofactor and for protein synthesis, photosynthesis, the synthesis of auxin, cell division, maintain of membrane structure [14]. Iron is involved in the production of chlorophyll, also is a component of many enzymes associated with energy transfer, nitrogen reduction and fixation, and lignin formation. Iron is associated with sulfur in plants to form compounds that catalyze other reactions. Manganese is necessary in photosynthesis, nitrogen metabolism and to form other compounds required for plant metabolism. Copper is necessary for carbohydrate and nitrogen metabolism and, inadequate copper results in stunting of plants. Copper also is required for lignin synthesis which is needed for cell wall strength and prevention of wilting[15].

Therefore, due to the sensitivity of petunia to nutrient availability and the obvious effect of these elements in the quality and quantity characteristics of flowers produced; in this study to improve the quantity and quality characteristics of Petunia is used spray of Humic acid.

MATERIALS AND METHODS

This study was carried out in completely randomized design with five treatments of humic acid (0, 100, 300, 600, and 900 ppm) and three replications on Petunia. Petunia seeds were planted at 3 kg pots filled with a 1:1 ratio of soil and leaf composts. First, four seed was planted in each pot and after germination and growth, the number of plants per pot was reduced to a plant. The seedlings at two-leaf stage 6 and 12 were treated with foliar application of humic acid (14%). Experimental parameters were measured 45 days after the second foliar.

Qualitative Characteristic

Fresh weight of root and shoot: stem of each plant was separated from the crown area and weight was measured by a digital scale with an accuracy of 0.01g (AND Scale EK, Japan). After removal of stems, roots of each plant was removed from the medium and gently washed by water pressure then weight measured.

Dry weight of root and shoot: isolated stems and roots after measurement the fresh weigh were placed in the oven at 60°C for 48 hours to dry and finally dry weight were measured by the digital scale with an accuracy of 0.01 g (AND Scale EK, Japan) .

Plant height, tillers and flowers per plant: Plant height was measured by a ruler with millimeter grading. Tillers and flowers are measured by means of enumeration of the number of generated tillers and appeared flowers per plant.

Leaf area index(LAI): All sampled leaves were scanned using a leaf area meter (YMJ-C, 20 TOP Instrument Inc., Hangzhou, China**)**. Average leaf area (mm^2) was calculated for each treatment.

Chlorophyll content: To calculate the chlorophyll content, five leaves per plant were selected and chlorophyll content was measured by chlorophyll meter(SPAD 502 Plus). The relationship between total chlorophyll content ($\mu g/cm^2$) and SPAD units was curvilinear and is expressed by the following equation [16]:

$$\mathrm{Chl} = \frac{117.1 \times \mathrm{SPAD}}{148.84 - \mathrm{SPAD}} \qquad R^2 = 0.89$$

Relative Water Content(RWC): relative water content of leaves were measured by soaking 0.2 g of leaf sample in 50 ml of distilled water for 4 hours by Whetherley (1950)[17]. After swelling, were weighed and then to determine the dry weight, leaves samples oven-dried at 70 ° C for 48 h and finally were weighed.

Before oven drying the samples at 80 °C, the tissues were air dried and placed in paper bags. After drying, the tissues were homogenised and ground. One gram from plant material were incinerated in an electric furnace at temperature of 550°C, then digested and extracted by 2N HCl. The prepared extract was used to measure the total concentration of Fe, Zn and Mn by Elmer Model 640 atomic absorption spectrophotometer [18]. Analysis of variance was performed with SPSS 16 software. Treatment means were compared with the Duncan's multiple range procedure. Statistical comparisons were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Data presented in tables 1 and 2 shows that the effect of humic acid treatments on all growth characteristics and micronutrient uptake were significant at 1% and 5% probability level. Based on data mean comparison (table3) HA_{600} treatment caused to the highest amount of fresh weight of shoot (105.3) and root (49) that showed significant difference with other treatment. As shown in Table 3, shoot and root dry weight showed no distinct significant differences between humic acid treatments, however there was significant statistical difference the among HA300 and HA600 treatments with control. HA_{600} treatment showed the highest chlorophyll content(11.6) and leaf area index (55) (table3). Table 3 shows that the mean measured characteristics height (41.8), number of tillers(11.6) and flowers (13.3) of Petunia hybrid at HA_{300} treatment was statistically significantly different from other treatments. Mean comparison of relative water content (RWC) trait in humic acid treatments showed no distinct significant difference, however, HA_{300} treatment in a compared to the control had significant increase in relative water content (82.3) (table 3). Data mean comparison of micronutrients uptake (Fe, Zn, Mn, Cu) by Petunia hybrid L. showed that the effect of different levels of humic acid on the absorption rate was statistically significant compared to controls. The highest concentrations of Fe, Mn and Cu were observed in HA_{300}treatment. Although, the mean Zn concentration was not significantly different among treatments, but the treatment of 300 ppm humic acid showed the highest concentration among the other treatments.

In general, the data in Table 3 show that the most effective humic acid treatment to improve the quality traits and absorption of micronutrients are HA_{300} and HA_{600} treatments.

Table 1-The results of analysis of variance on some measured quality traits

Source of variation	df	M.S of Chlorophyl	M.S of FWshoot	M.S of FWroot	M.S of DWshoot	M.S of DWroot	M.S of LAI	M.S of Hight	M.S of Tillers	M.S of Flowers	M.S of RWC
Treatment	4	27.3**	1301.5**	247.5**	68.7**	15.7**	519.6**	244.3**	22.0**	41.8**	140.7*
Error	10	1.4	23	10.9	15.6	3.2	13.6	9.0	2.1	3.8	49.2
Total	14										

Df= Degree of Freedom, ns= non-significant, *, **, significant at 0.05and 0.01 levels respectively

Table 2- The results of analysis of variance on some measured nutrients traits

Source of variation	df	M.S of Fe.leaf	M.S of Mn.leaf	M.S of Zn.leaf	M.S of Cu.leaf
Treatment	4	10062.1**	5609.7**	2428.2**	8.4*
Error	10	354.6	184.2	56.8	3.8
Total	14				

Df= Degree of Freedom, ns= non-significant, *, **, significant at 0.05and 0.01 levels respectively

Table 3-Effect of humic acid on the measured traits of *Petunia hybrid* L.

Treatment	FWshoot	FWroot	DWshoot	DWroot	Chlorophyll	LAI	Hight	Tillers	Flowers	RWC	Fe.leaf	Mn.leaf	Zn.leaf	Cu.leaf
HA_0	54.3c	24c	9b	6.3b	3.7d	35b	20.5c	4.6c	6.3b	65b	324.6c	103c	10.3b	6c
HA_{100}	85b	32.6b	16ab	8.3ab	6.5c	21c	31.3b	8.3b	7.6b	70ab	413.6b	144b	58a	9ab
HA_{300}	81.3b	38b	18a	10.6a	9.2b	41b	41.8a	11.6a	13.3a	82.3a	475a	193a	125a	12a
HA_{600}	105.3a	49a	22.5a	10a	11.6a	55a	29b	7.6b	14.6a	78.3ab	406.6b	84c	123a	10ab
HA_{900}	58.6c	34.3b	14.3ab	5.3b	9.1b	49a	19.8c	5.6bc	8b	75.6ab	454.6a	106c	125a	8b

Humic acid can stimulate shoot and root growth, and improve resistance to environmental stress in plants [19] but the physiological mechanism has not been well established.

Root development is due to not only the hormone-like effects of humic acid, but also is due to increased absorption of nutrients in the root. Humic acid due to increased nutrient uptake and hormone-like properties led to the increase of lateral roots of gerbera flowers[20]. In this study, the increase in fresh and dry weight of roots was observed. The researchers found that the application of humic acid (400 ppm) significantly increased the chlorophyll content [21,22].

Elkhateeb et al (2011) stated that the use of inoculative bio-fertilizers and humic acid significantly increased leaf area in acacia (*Acacia saligna*) as compared to the control [23]. Morard et al (2011) reported that humic acid increases yield and flowers nmuber in leaf area unit with nutrients absorption and its hormone-like properties which is in close agreement to our results [24]. HA produced visibly better and healthier plant growth and increased flower yield and quality of gerbera at 500 mg L^{-1} and economized water use in pomegranate (*Punica granatum* L.)[25,26], thereby reducing high operational costs and fertilizer application[26].

Studies have shown beneficial effects of HA such as increasing cell membrane permeability, oxygen uptake, respiration and photosynthesis, phosphate uptake, and root elongation. In particular, the photosynthetic efficiency and chlorophyll contents of Lolium perenne(rye-grass) were significantly increased by HA application [27].

Its substrate drenching or foliar application were equally effective in maintaining higher root fresh and dry weights in cucumber (*Cucumis sativus* L.), marigold, pansy (*Viola tricolor* L.), geranium (Pelargonium × hortorum L. H. Bailey), and impatiens (*Impatiens walleriana* Hook. f.) seedlings [28], and root growth and nutrient uptake in gerbera (*Gerbera jamesonii* Bolus ex Hooker f.) [26]. It increased the number of fruits and/or flowers, leaf area and plant height in *Triticum aestivum* L. [29,30]. Humic acid application was also beneficial for nutrient uptake, particularly uptake of N, P, K, Mg, Ca, Zn, Fe, and Cu by plants [26].

High pH values of soils which hinder the absorption of nutrients, also pose a problem for quality flower production. Foliar application is one of the methods to overcome this problem by providing nutrients necessary for optimal growth. Soil health is a crucial factor for obtaining higher yields of horticultural crops. Poor soil health and structure, and reduced microbial activities may result in poor crop stand, reduced plant growth and development [31]. However microelements as Fe, Zn, Mn and Cu are also added to foliar fertilizers used throughout the world as effective, preventive and curative measure to compensate their deficiency. It appears that absorption efficiency of the humic acid substances (HA) through young leaves via stomata was higher, and no accumulation on the surface of the leaf was observed and thus no scorching and burning of the leaf noted[32].

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REFERENCES

1. Dole, J.M. & Wilkins, H.F. [1999]. *Floriculture, Principles and Species*. Prentice Hall, New Jersey, 613 pp.
2. Simkin, A. J., Underwood, B. A., Auldridge, M., Loucas H. M., Shibuya, K., Schmelz, E., Clark D. G., **&** Klee H,J., [2004]. Circadian regulation of the PhCCD1 carotenoid cleavage dioxygenase controls emission of beta-ionone, a fragrance volatile of petunia flowers, Plant Physiol.;136(3):3504-14.
3. Folegatti, M. V., Blanco F. F; Boaretto, R. M., **&** Boaretto, A. E., [2005]. Calibration of cardy-ion meters to measure nutrient concentrations in soil solution an in plant sap, Sci. Agric. (Piracicaba, Braz.), 62: 8-11
4. Romemheld, V., **&** El-Fouly, M.M. [1999]. Foliar nutrient application, Challenge and limits in crop production, Proc. 2nd International Workshop on "Foliar Fertilization" April 4-10 Bangkok, Thailand. pp: 1-32.
5. Saqib, M., Zörb, C & Schubert, S. [2006]. Salt-resistant and salt-sensitive wheat genotypes show similar biochemical reaction at protein level in the first phase of salt stress, Journal of Plant Nutration and Soil Science. 169: 542-548.
6. Michael H. B. H., **&** Ronald L. M., [2001]. Considerations of Compositions and Aspects of the Structures of Humic Substances, in book: Humic Substances and Chemical Contaminats, p: 3-39
7. Senn T.L., [1991]. Humates in Agriculture, Acres USA, Jan.
8. Fu Jiu C., Dao Qi Y., & Quing Sheng W. [1995]. Physiological effects of humic acid on drought resistanceof wheat (in Chinese), Yingyong Shengtai Xuebao 6: 363–367.
9. Malik K.A., & Azam F., [1985]. Effect of humic acid on wheat (Triticum aestivumL.) seedling growth, Environ. Exp. Bot. 25: 245–252
10. Figliolia A., Benedetti A., Izza C., Indiati R., Rea E., Alianiello F., Canali S., Biondi F.A., Pierandrei F., & Moretti R. [1994]. Effects of fertilization with humic acid on soils and plant metabolism: A multidisciplinary approach. Note I: Crop production, in: Humic Substances in the Global Environment and Implications on Human Health-Proceedings of the 6th International Meeting of the International Humic Substances Society, Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 579–584.
11. Piccolo A., Nardi S., & Concheri G. [1992] Structural characteristics of humic substances as regulated tonitrate uptake and growth regulation in plant systems, SoilBiol. Biochem. 24: 373–380
12. Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I & Lux, A. [2007]. Zinc in plants. New Phytol. 173: 677– 702
13. Medina, J., Clavero-Ramı´rez, I., Gonza´lez-Benito, M., Ga´lvez-Farfan, J., Manuel Lo´pezAranda, J., & Soria, C [2007]. Field performance characterization of strawberry (Fragaria ananassa Duch.) Plants derived from cryopreserved apices. Scientia Horticulturae.113: 28–32.
14. Marchner, H. [1995]. Mineral nutrition of higher plants. 2nd ed. Academic Press. London.
15. Allen V. Barker, David J. Pilbeam [2007]. Handbook of Plant Nutrition, CRC PressTaylor & Francis Group
16. Coste, S., Baraloto, C., Leroy, C., Marcon, É., Renaud, A., Richardson, A. D., ean-hristophe Roggy, J. C., Schimann, H., Uddling, J., & H´erault, B., [2010]. Assessing foliar chlorophyll contents with the SPAD-502 chlorophyll meter: a calibration test with thirteen tree species of tropical rainforest in French Guiana, Ann. For. Sci. 67 : 607
17. Whetherley, P.E., [1950]. Studies in water relations of cotton plants I. The field measurement of water deficit in leaves. New Phytol. 49: 81-87.

18. Wright, R. J & Stuczynski T. I. [1996]. Atomic absorption and flame emission spectrometry. In: Methods of Soil Analysis, Part 3: Chemical Methods., Sparks, D. L. ed., SSSA Book Series Number 5, Soil Science Society of America, Madison, WI ,pp65-90.
19. Goatley, J. M. Jr., & Schmidt, R. E. [1990]. Anti-senescence activity of chemicals applied to Kentucky bluegrass. J. Am. Soc. Hortic. Sci. 115,57–61.
20. Autio, J. [2000]. Supplementary lighting regimes strongly affect the quantity of gerbera flower yield. Acta Hort. 515:91-98.
21. Liu, C. H., Cooper, R. J., & Bowman, D. C. [1998]. Humic acid application affects photosynthesis, root development, and nutrient content of creeping bentgrass. HortScience 33: 1023-1025.
22. Neilsen, G. H., Hogue, E.J., Neilsen, D., & Bowen, P. [2005]. Postbloom humic and fluvic- based zinc sprays can improve apple zinc nutrition. HortScience 40:205-208.
23. El-Khateeb, M. A., El-Leithy, A. S. **&** Aljemaa, B. A. [2011]. Effect of mycorrhizal fungi inoculation and humic acid on vegetative growth and chemical composition of acacia salignaLabill. Seedlings under Different Irrigation Intervals, Journal of Horticultural Science & Ornamental Plants 3 (3): 283-289
24. Morard, P., Eyheraguibel, B., Morard, M., **&** Silvestre, J. [2011]. Direct effects of humic-like substance on growth, water, and mineral nutrition of various species. J. Plant Nutr. 34(1):46-59.
25. Khattab, M. M., Shaban, A. E., El-Shrief, A. H., **&** El Deen Mohamed, A. S. [2012]. Effect of humic acid and amino acid on pomegranate trees under deficit irrigation. I: Growth, flowering, and fruiting. Journal of Horticultural Science & Ornamental Plants 4:253-259
26. Nikbakht, A., Kafi, M., Babalar, M., Xia, Y.P., Luo, A., **&** Etemadi, N. [2008]. Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera. Journal of Plant Nutrition 31:2155-2167.
27. Russo, R.O., **&** Berlyn, G.P. [1990]. The use of organic biostimulants to help low input sustainable agriculture. Journal of Sustainable Agriculture 1:19-42
28. Li, G., & Evens, M.R. [2000]. Humic acid substrate treatments and foliar spray application effects on root growth and development of seedlings. HortScience 35:434
29. Chen, Y., Clapp, C.E., & Magen, H. [2004a]. Mechanism to the plant growth stimulation by humic substances: the role of organo-iron complexes. Soil Science and Plant Nutrition 50:1089-1095.
30. Chen, Y., De Nobili, M., & Avaid, T. [2004b]. Stimulatory effect of humic substances on the plant growth. p. 103. In: Magdoff, F., & Weil, R.R. (eds.) Soil and organic matter in sustainable agriculture. CRC Press, Washington, D.C., USA.
31. Baldotto, M.A., & Baldotto, L.E.B. [2013]. Gladiolusdevelopment in response to bulb treatment with different concentrations of humic acids. Revista Ceres 60:138-142.
32. Kaya, M., Atak, M., Khawar, K. M., Çiftçi C. Y., & Özcan, S., [2005]. Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of common bean (Phaseolus vulgarisL.), Int. J. Agri. Biol., 7: 875-878

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